12. Fieser, L. F., and Fieser, M., *Organic Chemistry,* D. C. Heath & Co., Boston, 1944.

13. Jakson, E. L., in *Organic Reactions,* vII, John Wiley & Sons, New York, 1944.

14. Pfiffner, J. J., and North, H. B., *J. Biol. Chem.,* 1940, v132, 459.

15. Cuyler, W. K., Hirst, D. V., Powers, J. M., and Hamblen, E. C., *J. Clin. Endocrinol.,* 1942, v2, 373.

16. Pearlman. W. H., in *The Hormones,* vI, New York Acad. Press, 1948.

17. a. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Arch. Int. Med.,* 1950, v85, 199. b. Sprague, R. G., Mason, H. L., Mathieson, D. R., Bennett, A., and Gastinean, C. F., in Proc. II. Clinical ACTH Conf., v2, The Blakiston Co., 1951.

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Separate Identities of the Donath-Landsteiner Hemolysin (PCH Antibody) and Treponemal Immobilizing Antibody.* (19621)

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Paroxysmal cold hemoglobinuria (PCH) due to the presence of an abnormal serum hemolysin is recognized as a manifestation of syphilis, for nearly all patients with positive Donath-Landsteiner reactions have historical, clinical or serological evidence of syphilitic infection(1-3). The hemolysin is distinct from the Wassermann reagin(4,5), and surveys of non-hemoglobinuric patients with syphilis have detected only a few with positive Donath-Landsteiner reactions(6-8).

Since demonstration of the PCH hemolysin sometimes requires more complement than was used in the tests employed for these surveys (9), it was felt that a more sensitive test might reveal that many patients with syphilis have a detectable, but clinically inactive, hemolysin. Furthermore, because both the PCH antibody (10) and the treponemal immobilizing (TPI) antibody(11,12) are heat stable globulins that require complement for their activities, and because TPI antibody is a specific indicator of syphilitic infection, the relationship of these antibodies was investigated in an effort to clarify further the mechanism of production of syphilitic cold hemoglobinuria.

Materials and methods. Sera. For survey purposes, sera were obtained from hospitalized and clinic patients with strongly positive Wassermann and Kline reactions. The sera used as controls in the absorption experiment were obtained from patients who were not receiving penicillin or other antibiotics. The case histories of the 2 patients with paroxysmal cold hemoglobinuria have been summarized elsewhere(8). *Antibody tests.* The methods used for the following procedures were those previously described(8): 1) hemolysin titration, 2) indirect Coombs tests (a) and (b). The technic of Nelson and Diesendruck(13) was employed for the treponemal immobilization test.† *Absorption of sera.* Sera from the 2 patients with positive Donath-Landsteiner reactions and from 2 other patients with syphilis (controls), but with negative hemolysin reactions, were treated as follows:

Three ml of serum, 0.75 ml of freshly reconstituted lyophilized guinea pig serum, and 1.5 ml of fresh, thrice washed packed group O erythrocytes were added to a tube. This mixture was chilled in an ice bath for an hour, centrifuged in the cold, and the supernatant removed for testing. The erythrocytes used for absorption were washed 3 times with cold saline and tested for agglutination by antiglobulin serum in the indirect Coombs test (a). For each serum, 3 specimens, identified

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TABLE I. PCH Antibody Titers in Sera before and after Absorption.

Case	Specimen	Test	Initial dilution of serum: Undiluted	2	4	8	16	32	64
1	#2	H*	3+	3+	3+	3+	2+	+	0
	Unadsorbed	A†	3+	3+	3+	2+	2+	2+	+
	#3	H	+	+	0	0	0	0	0
	Adsorbed	A	3+	2+	2+	0	0	0	0
2	#2	H	3+	2+	+	0	0	0	0
	Unadsorbed	A	2+	3+	2+	+	0	0	0
	#3	H	0	0	0	0	0	0	0
	Adsorbed	A	0	0	0	0	0	0	0

* Hemolysis in Donath-Landsteiner reaction.
† Agglutination in indirect Coombs test (b).

TABLE II. Effect of Absorption of PCH Antibody on Treponemal Immobilization Capacity of Syphilitic Sera.

	Specimen No.*	% Treponema motile: With inactive C′	With active C′	Result	Residual complement
Case 1	1	80			
	2	80			
	3	72			
2	1	76			
	2	92			
	3	88	0	+	4+
Control 1	1	100			
	2	88			
	3	88			
2	1	84			
	2	84			
	3	88			

* Specimen No. 2 was absorbed (see text).

by number and complement content, were frozen and submitted for the TPI test. Specimen No. 1 was 1.25 ml of untreated serum; No. 2 was 1.25 ml of the absorbed serum and complement mixture, representing 1 ml of serum and .25 ml of reconstituted lyophilized guinea pig serum; No. 3 consisted of 1.0 ml of serum plus 0.25 ml of guinea pig serum.

Results. Survey. Sera from 33 patients with serological evidence of syphilis were titrated for hemolysin and indirect PCH antibody. No additional individuals with positive Donath-Landsteiner reactions were found.

Absorption. Only those erythrocytes used to absorb sera from patients with hemoglobinuria were agglutinated by antiglobulin serum. The effect of absorption on PCH antibody is shown in Table I.

Table II indicates that absorption of patient and control sera did not alter the treponemal immobilizing capacity of the sera. Thus, PCH antibody may be removed from sera without removing TPI antibody. The reverse experiment could not be performed because absorption of TPI antibody is not yet feasible (14).

Discussion. Paroxysmal cold hemoglobinuria in syphilitic patients is associated with a positive Donath-Landsteiner reaction. Conversely, patients with positive Donath-Landsteiner tests nearly always, if not always, have syphilis. Although syphilitic infection is responsible for the hemoglobinuria, very few patients with syphilis develop an autohemolysin. Cold hemoglobinuria in non-syphilitics can be attributed to the presence of potent cold hemagglutinins(3,15).

The low incidence of positive tests for PCH antibody as an indication of syphilitic infection (even utilizing a more sensitive test) is in contrast to the high incidence of positive Wassermann and treponemal immobilization

tests. Both PCH(5) and TPI(11) antibodies have been shown to be distinct from the Wassermann reagin. Although both are globulins, the PCH antibody is a *gamma*-globulin(10) while the spirocheticidal TPI antibody appears to be associated with the *beta*-globulins (12). The present study has demonstrated that reduction or removal of PCH antibodies by absorption fails to alter the qualitative TPI reaction. Quantitative TPI titrations were not feasible(14), and it is possible that TPI antibody might have been removed without changing the outcome of a qualitative test. This is considered to be unlikely. The absorption results support the serum fractionation studies in indicating that the 2 types of antibody are not related, although final proof must await the development of a method for reciprocal absorption of the TPI antibodies. TPI antibodies appear as a specific response to infection with *Treponema pallidum*(11). The mechanism of development and the low occurrence of the PCH hemolysin in patients with syphilis remain unexplained.

Summary. A more sensitive Donath-Landsteiner test did not detect the hemolysin of paroxysmal cold hemoglobinuria in sera of patients with syphilis more often than tests used in other surveys. Although related to syphilitic infection, PCH antibody was shown to differ from treponemal immobilizing antibody, the specific antibody of this infection. The mechanism of production of PCH antibody remains unexplained.

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1. Donath, J., and Landsteiner, K., *Ergebrn. d. Hygiene,* 1925, v7, 184.
2. MacKenzie, G. M., *Medicine,* 1929, v8, 159.
3. Becker, R. M., *Arch. Int. Med.,* 1948, v81, 630.
4. Smith, R. P., *J. Path. and Bact.,* 1923, v26, 196.
5. MacKenzie, G. M., *J. Clin. Invest.,* 1929, v7, 27.
6. Donath, J., and Landsteiner, K., *Z. f. klin. Med.,* 1905, v58, 173.
7. Kumagi, T., and Namba, M., *Deutsches Arch. f. klin. Med.,* 1927, v156, 257.
8. Dill, L. V., *Am. J. Syphilis, Gonorrhea, and Venereal Dis.,* 1939, v23, 220.
9. Jordan, W. S., Jr., Pillemer, L., and Dingle, J. H., *J. Clin. Invest.,* 1951, v30, 11.
10. ———, *J. Clin. Invest.,* 1951, v30, 22.
11. Nelson, R. A., Jr., and Mayer, M. M., *J. Exp. Med.,* 1949, v89, 369.
12. Tauber, H., McLeod, C., Garson, W., and Magnuson, H. J., *Fed. Proc.,* 1951, v10, 258.
13. Nelson, R. A., Jr., and Diesendruck, J. A., *J. Immunol.,* 1951, v66, 667.
14. Mayer, M. M., Personal communication.
15. Stats, D., and Wasserman, L. R., *Medicine,* 1943, v22, 363.

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Inhibitory Effect of Antibiotics on Virus of Rous Sarcoma. (19622)

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Rous(1) described a sarcoma of chickens which was apparently caused by a filterable virus. This tumor is a spindle-shaped growth which is both infiltrative and destructive. The tumor can be transmitted by cell-free filtrates, by desiccated tumor tissue and by glycerine extracts. The tumor can also be transmitted by the blood, ascitic fluid and tumor-free organs of the tumor bearing animal providing the tumor is of considerable size. The more recent work of Porter and Thompson(2) and others on electron microscope examination of the tissue of the Rous sarcoma shows the presence of virus-like particles which are believed to be the etiological agent of this tumor.

This report deals with the effect of certain antibiotics on the virus of the Rous sarcoma of chickens. Penicillin, streptomycin, bacitracin, terramycin, chloramphenicol, aureomycin, neomycin and antibiotic PA96(Pfizer) were tested. Various concentrations of each